



Summary of Ph.D. thesis

# **The *Drosophila melanogaster* calpains and the border cell migration**

Ferencz Sándor Páldy

Supervisors: Prof.Dr. János Gausz  
Dr. Géza Ádám

Doctoral School of Biology  
University of Szeged  
Faculty of Science and Informatics  
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## Introduction

For the normal function of living organisms, the delicate balance between the anabolic and catabolic processes is of major importance. Proteolysis plays a major role in tissue reorganization and regeneration, immunity, cell cycle regulation, metastasis etc.

As a consequence of proteolytic activity, the targeted substrate molecules can be degraded completely or just partially (regulatory function). Proteolytical enzymes are a large and extremely heterogeneous group of proteins, which can be classified according several different aspects. According to the nature of its catalytic aminoacid(s), there are cysteine -, serine -, treonine -, asparagine -, aspartate - and metalloproteases. According to the location of the cleavage site on the target molecule, there are endo-, exo- and omegapeptidases. Abnormal or nonfunctional enzymes are linked to severe illnesses, like: cancer (MMP9, MMP12), malaria, type 2 diabetes, Alzheimer's disease etc.

Calpains are cytosolic, neutral (pH 7.1-7.3) and  $\text{Ca}^{2+}$ -activated cysteine proteases. Calpain gene numbers increase with the growing of the complexity of organisms. In *D. melanogaster* we can find 4 calpain genes (*CalpA*, *CalpB*, *CalpC*, *CalpD*). From these 4 genes, only 2 code for a both functional and canonical calpain (*CalpA*, *CalpB*). A functional calpain contains all 3 catalytically active residues

(Cys, His, Asn) and typically contains 4 protein domains (DI, DII, DIII, DIV).

Based on cell culture assays (CHO cells, mouse embryonic fibroblasts), the link between cell migration and calpains has been previously established. Using this as a starting point, we analyzed the possible roles of *D. melanogaster* calpains in cell migration.

## Objectives

- comparison of the mammalian calpains with the *D. melanogaster* calpains
- find out what are the roles of the *D. melanogaster* calpains (*CalpA*, *CalpB*)
- analyze the possibility of functional redundancy between these genes
- study the effects of calpains on cell migration *in vivo*
- find genetic interaction partners
- dissect possible mechanism

## Methods

- Ovary preparation and staining
- Cloning
- PCR
- RT-PCR
- SDS-Page and Western blot
- Immunoprecipitation
- Cleavage of talin *in vitro*
- General calpain activity measurement

## Results

### **a.** The *Drosophila melanogaster* calpains are not essential.

Since some of the mammalian calpains are essential, we expected to achieve similar results in the fruit fly too. In contrast with our expectations, none of the examined *Drosophila* calpains proved to be vital.

To create deletion mutants, we remobilized P-elements found in the *CalpA* and *CalpB* genes. In the case of *CalpA*, the

remobilization of  $p\{SUPor-P\}KG^{05080}$  transposon resulted in the  $CalpA^{808}$  mutant, carrying a 2.490 bp deletion. This affected not only the  $CalpA$ , but also the neighboring  $hts$  gene and resulted in female sterility, due to the  $hts$ . This phenotype was rescued, when a larger segment of  $hts$  was reintroduced in the flies. In the  $CalpA^{808}$  mutant neither RNA transcription nor protein translation could be observed. In the case of  $CalpB$  we remobilized the  $p\{EPgy2\}EY^{08042}$  transposon, generating several deletion mutants ( $CalpB^{505}$ ,  $CalpB^{502}$ ,  $CalpB^{392}$ ,  $CalpB^{389}$ ,  $CalpB^{361}$ ). The  $CalpB^{505}$  carried a 1.047 bp -, while the  $CalpB^{361}$  a 922 bp deletion. In both cases the transcription of truncated RNAs could be observed, but protein translation didn't occur from these. Deletion of the  $CalpA$  and  $CalpB$  genes didn't affect the viability or fertility of the flies.

**b. Lack of  $CalpB$  affects the general level of calpain activity.**

To measure the calpain activity in  $CalpB$  mutants, we used a synthetic calpain substrate, the  $Dabcyl$ -TPLKSPPPSPRE- $EDANS$ . In the uncut substrate molecule, the fluorescent signal emitted by the  $EDANS$  segment is quenched by the  $Dabcyl$  domain (FRET). When digestion occurs, the quenching takes no longer place, therefore the fluorescent signal will increase. By this method we found, that in both  $CalpB$  mutants ( $CalpB^{505}$ ,  $CalpB^{502}$ ) analyzed, the calpain

activity level dropped to 50% of the original value.

**c. CalpB function affects border cell migration.**

Even if the deletion of the *CalpA* and *CalpB* genes didn't affect viability and fertility, we couldn't exclude any structural and/or functional changes that do not present any visible changes to the outside. Previous studies conducted on cell lines (mouse embryonic fibroblasts, CHO), already established the link between calpains and cell migration. Based on this information, we analyzed whether our deletional mutants present any phenotypic changes in the border cell migration. In the *CalpA*<sup>808</sup> mutants we didn't observe any change in the migration of the border cells. On the other hand, in the case of *CalpB*, we did find a significant reduction in the migration rate of the border cells. In both the *CalpB*<sup>505</sup>, *CalpB*<sup>361</sup> mutants, 30-40% of the observed egg chambers showed a delayed migratory phenotype. The *CalpA-CalpB* double mutants, didn't show any aggravation of the phenotype and it could be rescued by reintroducing the wild-type *CalpB* in in the flies.

To backup our results with an independent method, we performed RNA<sub>i</sub> (RNA interference). Using a driver specific for the border cells (*slbo-Gal4*), we silenced the *CalpA* and *CalpB* genes with gene specific RNA<sub>i</sub>

constructs publicly available. The results of these experiments were similar to our previous findings with the deletional mutants.

**d. The *CalpA* and *CalpB* genes are not functionally redundant.**

This conclusion is substantiated by the results, that the *CalpA-CalpB* double mutants viability and fertility was not affected. Also, the delayed border cell migration phenotype was unaltered and remained at the 30-40% mutant rate caused by the deletion of *CalpB*.

**e. *CalpB* interacts with focal adhesion components.**

We focused our attention on 3 focal adhesion components: the  $\beta$ -PS-integrin encoded by the *mysospheroid* gene (*mys*), the  $\alpha$ -PS2 integrin coded by the *inflated* gene (*if*) and the talin coded by the *rhea* gene. We found, that with all 3 studied focal adhesion components the *CalpB* showed a strong genetic interaction. This resulted in an elevated penetrance, since the original level of 30-40% delayed border cell migration increased to 58-75%, depending on case.

**f. *CalpB* releases integrins by cleaving talin.**

Previous studies have shown, that calpains cleave talin. Therefore we examined, whether this cleavage occurs in *Drosophila* too. Using immunoprecipitation we found, that talin

copurified with CalpB, proving that there is a direct physical contact between the proteins. SDS-Page and Western blot analysis showed, that talin is cleaved in the presence of CalpB.

Under normal conditions, behind the group of migratory border cells, integrin is released. This can be easily tracked, since a trail of integrin spots are left behind. In the *CalpB* mutants, this integrin release is halted. Talin is known to stabilize the integrin's connection to the actin cytoskeleton. The opposite end of an integrin molecule connects to the extracellular surface on which the cells migrate. Therefore, it is plausible if upon calpain cleavage of talin, this stabilization effect disappears and integrin is released into the extracellular space. This results in normal migration rates. When CalpB is deleted, talin is not cleaved anymore and therefore the link between integrins and actin cytoskeleton stabilizes, leading to a slower focal adhesion turnover rate and to a decrease in the migration rates of the border cells.



## Publications

1. Kókai E., Páldy<sup>\*†</sup> F. S., Somogyi K., Chougule A., Pál M., Kerekes É., Deák P., Friedrich P., Dombrádi V., Ádám G. CalpB modulates border cell migration in *Drosophila* egg chambers. *BMC Dev Biol* **12**, 20 (2012).

IF 2.79

2. Lovdok L., Bentele K., Vladimirov N., Müller A., Pop F. S., Liebidz D., Kollmann M., Sourjik V. Role of translational coupling in robustness of bacterial chemotaxis pathway. *PLoS Biol* **7** (2009).

IF 12.91

\* - change of last name (Páldy, born Pop)

† - shared first authorship

## Posters

1. VI. Hungarian Genetics Congress and XIII. Cell- and Developmental Biology Days, 2005, Eger, Hungary

## Oral presentations

1. 12<sup>th</sup> Regional Drosophila Meeting, 2006, Vienna, Austria
2. VII. Hungarian Genetics Congress and XIV. Cell- and Developmental Biology Days, 2007, Balatonfüred, Hungary
3. VII. Genetics Workshop in Hungary, Minikonference, 2008, Szeged, Hungary